Pru p 3 acts as a strong sensitizer for peanut allergy in Spain

Pru p 3 has been suggested to be the primary sensitizing allergen in patients with peanut allergy in the Mediterranean area.[1,2] We aimed to confirm this hypothesis, studying 79 subjects (median age, 28 years; age range, 25-35 years; 61% female). According to a clinical history of reactions within the last 2 years, 51 had peanut allergy with a positive skin prick test (SPT) response. Twenty-eight (sensitized group) had positive peanut SPT responses with no symptoms on peanut intake, as confirmed by an open challenge (20 g of roasted peanuts; Casa Pons, Importaco, Valencia, Spain). The timing of symptom onset with peanut, peach, and hazelnut was also evaluated. Hazelnut and peach allergy were based on history and SPT responses. All demographic and clinical data are shown in Table E1 in this article's Online Repository at www.jacionline.org. This study was approved by the ethics committee (trial no. 032/2009, March 9, 2009).

SPTs were performed on all patients with standardized protein extracts from peanut, hazelnut (Bial-Aristegui, Bilbao, Spain), and peach (30 μg/mL Pru p 3; ALK-Abelló, Madrid, Spain). Wheals of 3 mm in diameter were considered positive.

Levels of specific IgE (sIgE) against rAra h 1, rAra h 2, rAra h 3, rAra h 8, rAra h 9, rPru p 3, and rCor a 8 were measured by using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden), according to the manufacturer's instructions. Serum sIgE values were quantified in kilounits of allergen (kU_A) per liter, with values of 0.35 kU_A/L or greater considered positive. Values of less than 0.1 kU_A/L were considered 0.

We performed ELISA cross-inhibition with sera from patients with peanut allergy (n = 26) who had sIgE to all 3 nonspecific lipid transfer proteins (LTPs) analyzed (rAra h 9,[3] rPru p 3,[4] and rCor a 8[5]). These allergens were bound to the solid phase and used for inhibition with the individual sera at different concentrations (20, 10, 5, and 0.5 μg/mL).[6]

Quantitative variables are shown as medians and ranges, and qualitative variables are presented as frequencies (percentages). Medians between groups were compared by using Mann-Whitney *U* or Kruskal-Wallis tests, whereas the χ^2 test (or the Fisher exact test, when needed) was used to compare proportions. Statistical analyses were performed with SPSS version 15.0 software (SPSS, Chicago, Ill). Significance was set at a *P* value of less than .05.

Most of the patients with peanut allergy (n = 42 [82.4%]) had sIgE against Ara h 9 (median, 3.43 kU_A/L; range, 0-100 kU_A/L), with very few positive cases against the major peanut allergens Ara h 1 (n = 1; median, 0 kU_A/L; range, 0-6.48 kU_A/L), Ara h 2 (n = 2; median, 0 kU_A/L; range, 0-7.07 kU_A/L), Ara h 3 (n = 2; median, 0 kU_A/L; range, 0-1.46 kU_A/L), or Ara h 8 (n = 3; median, 0 kU_A/L; range, 0-5.18 kU_A/L). No differences were found in these IgE levels regarding symptom severity.

Among the sensitized group, we found positive sIgE levels against Ara h 9 in 21 subjects (median, 1.34 kU_A/L; range, 0-95.2 kU_A/L), against Ara h 1 in 2 subjects (median, 0 kU_A/L; range, 0-0.46 kU_A/L), against Ara h 2 in 1 subject (median, 0 kU_A/L; range, 0-1.38 kU_A/L), against Ara h 3 in 3 subjects (median, 0 kU_A/L; range, 0-0.57 kU_A/L), and against Ara h 8 in 3 subjects (median, 0 kU_A/L; range, 0-1.8 kU_A/L). Both sIgE levels and sensitization frequency to Ara h 9 were similar between patients allergic to peanut and those sensitized to peanut (χ^2 test, $P = .437$; Mann-Whitney *U* test, $P = .152$), confirming that Ara h 9 is the main allergen in Spanish peanut-sensitized patients.[1,2]

When analyzing all the subjects, the sensitization frequency against the other LTPs was high (83% of patients sensitized to Pru p 3: median, 4.5 kU_A/L [range, 0-100 kU_A/L]; 66% of patients sensitized to Cor a 8: median, 0.9 kU_A/L [range, 0-67.9 kU_A/L). Although peanut LTP sensitization was similar in both patients with peanut allergy and patients with peanut sensitization, the number of positive IgE results against Pru p 3 was higher in patients with peanut allergy (90%) than in peanut-sensitized patients (71%; $P = .054$, Fisher test). Cor a 8 sensitization was also higher in patients with peanut allergy (74%) than in peanut-sensitized patients (50%; $P = .028$, Fisher test). Being allergic to peanut was associated with the development of symptoms on peach ingestion ($P < .001$, χ^2 test; odds ratio, 7.5 [95% CI, 2.4-23.6]) and also having symptoms with hazelnut ($P = .001$, χ^2 test; odds ratio, 5.9 [95% CI, 1.9-18.4]). Only 1 patient (without symptoms to peanut) was reactive to Ara h 9 but not to Pru p 3. In contrast, the patient collective includes 5 subjects reactive to Pru p 3 (all with symptoms to peach) without cosensitization to Ara h 9.

Interestingly, among the 45 patients sensitized to peanut with peach allergy, 33 showed that symptoms with peach appeared before peanut allergy, whereas none of the patients with peanut and hazelnut allergy (n = 27) became allergic to peanut after being allergic to hazelnut. Two thirds (18/27) of the patients with peanut allergy who also presented symptoms to hazelnut became allergic to peanut and later to hazelnut, according to the clinical data. Because these are retrospective data, performing prospective studies to explore whether peach allergy leads to peanut sensitization is recommended.

Our data suggest that both peanut and hazelnut sensitization are related to peach allergy in an important number of cases. When analyzed by using cross-inhibition studies, 20 of 26 patients with peanut allergy with sIgE against Ara h 9, Pru p 3, and Cor a 8, Pru p 3 had a similar or stronger inhibitory capacity to Ara h 9 and Cor a 8 than Ara h 9 (Fig 1, *A*). We observed no stronger capacity of Cor a 8 or Ara h 9 to inhibit IgE to Pru p 3 than with Pru p 3. This indicated that Pru p 3 was the primary sensitizer. Six of 26 patients had Ara h 9 primary antibodies (Fig 1, *B*). The strongest inhibition to Ara h 9 was obtained by using self-inhibition, and neither Pru p 3 nor Cor a 8 reached the same value. Similar experiments with Cor a 8 showed no evidence for primary antibodies to this allergen. Comparison of inhibitions at inhibitory concentration for 25% inhibition (IC_{25}) values are shown in the Methods section in this article's Online Repository at www.jacionline.org. Only 1 patient (Fig 2) had primary antibodies to Ara h 9, with even higher inhibitory capacity of IgE binding to Pru p 3 in the solid phase than Pru p 3.

These findings demonstrate that Pru p 3 is the predominant allergen in most of our patients, with this LTP being the main sensitizer in patients with peanut allergy, as has been reported by others.[1,7] Cor a 8 displayed lower inhibition compared with peach

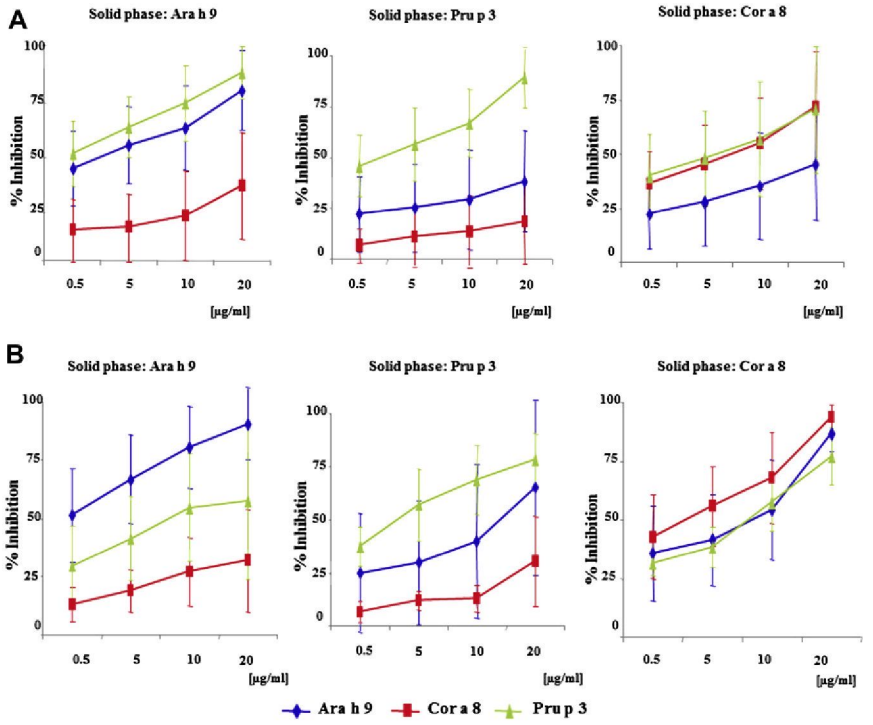

FIG 1. Inhibition ELISA with Pru p 3, Ara h 9, and Cor a 8 immobilized in the solid phase and 4 concentrations (20, 10, 5, and 0.5 µg/mL) of these allergens as inhibitors in the fluid phase. Inhibition percentages of the IgE binding against the different solid phases when using the same allergens are represented as means and SDs of inhibition values. **A,** Inhibition percentage of the IgE binding of the patients (n = 20) with primary sensitization to Pru p 3. **B,** Inhibition percentage of the IgE binding of the patients (n = 6) with primary sensitization to Ara h 9.

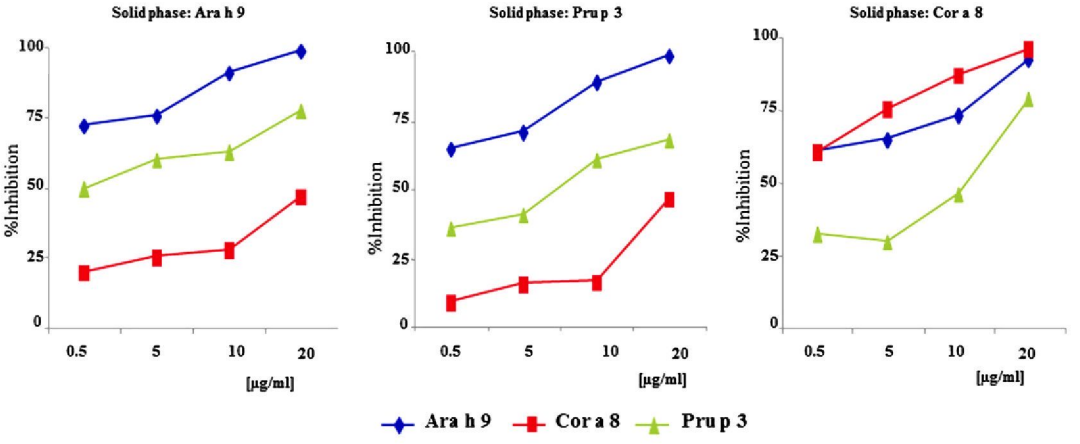

FIG 2. Inhibition ELISA with Pru p 3, Ara h 9, and Cor a 8 immobilized in the solid phase and 4 concentrations (20, 10, 5, and 0.5 µg/mL) of these allergens as inhibitors in the fluid phase. Percentages of inhibition of the IgE binding against the different solid phases when using the same allergens are represented.

LTP, as has been reported,[8] and also with peanut LTP. We conclude that being allergic to peach can be a risk factor for becoming allergic to peanut. Patients with peanut allergy must be followed up and investigated for sensitization and symptom challenges with peach, hazelnut, and peanut to clarify the importance of previous peach allergy on becoming allergic to peanut. From the diagnostic viewpoint, in those areas where Rosaceae fruits are widely consumed, in patients with peanut allergy, peach allergy should be considered a primary sensitization, and therefore testing Pru p 3 is recommended in those patients.

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METHODS

Calculation of inhibitory concentration

We calculated the IC_{25} value of each allergen with the sera studied. We then estimated the number of sera with Ara h 9 IC_{25} values greater than, less than, or similar to the IC_{25} value of Pru p 3 for both allergens in the solid phase. Cor a 8 values were not calculated because of the lower inhibitory capacity.

We then classified the sera (n = 26) according to the IC_{25} values, obtaining 2 groups:

- *Group with primary antibodies against Pru p 3*: In these sera we found that Pru p 3 was the most potent inhibitor when this allergen was bound to the solid phase (IC_{25} Pru p 3 [mean, 0.348 μg/mL] < IC_{25} Ara h 9 [mean, 11.7 μg/mL]). When the solid phase used was Ara h 9, the IC_{25} values of Pru p 3 were similar to those of Ara h 9, with the mean IC_{25} value for the first being 0.305 μg/mL and that for the second being 0.345 μg/mL. This group included 20 subjects.
- *Group with primary antibodies against Ara h 9 and cross-reactivity with Pru p 3*: We found that Ara h 9 was the most potent inhibitor when this allergen was also bound to the solid phase (IC_{25} Ara h 9 [mean, 0.317 μg/mL] < IC_{25} Pru p 3 [mean, 7.53 μg/mL). However, when the Pru p 3 was bound to the solid phase, the inhibitor Pru p 3 in the fluid phase inhibited the most (IC_{25} Pru p 3 [mean, 0.358 μg/mL] < IC_{25} Ara h 9 [mean, 15.86 μg/mL). This group included 6 subjects. These findings indicate that co-existing antibodies exist in these subjects, one primary antibody directed to Ara h 9 with cross-reactivity with Pru p 3 and to a lesser degree with Cor a 8 and primary antibodies to Pru p 3 with a different degree of cross-reactivity to Ara h 9 and Cor a 8.

Calculations made at 50% inhibition and a higher inhibition (50 μg/mL) than the one shown in the graphs (20 μg/mL) produced values that were still less than 100%, with the allergens used in inhibition being different from the allergen bound to the solid phase.

TABLE E1. Summary of clinical characteristics and sensitization profiles (SPT responses and sIgE levels against the allergens studied) of the patients, both those with peanut allergy and sensitized tolerant patients (n = 79)

Subject no.	Age (y)	Sex	Symptoms			SPT response			sIgE level (ImmunoCAP, kU_A/L)						
			Peanut	Hazelnut	Peach	Peanut	Hazelnut	Peach	Ara h 1	Ara h 2	Ara h 3	Ara h 8	Ara h 9	Cor a 8	Pru p 3
1	15	M	None	Never eaten	None	+	+	+	0	0	0	0	95.2	40.2	100
2	15	F	None	None	None	+	−	+	0	0	0	0	12.9	0.19	5.43
3	64	F	None	U	None	+	−	−	0	0	0	0	0	0	0
4	66	M	None	None	None	+	+	+	0	0	0.37	0.95	0	0	0
5	18	M	None	None	None	+	+	−	0.46	0.15	0.13	0	0	0	0.19
6	28	F	None	U	U	+	+	+	0	0	0	0	1.93	0.63	2.53
7	28	F	None	U	U	+	+	+	0	0	0	0	76.1	9.95	90.8
8	23	M	None	None	OAS	+	+	+	0	0	0	0	1.49	0.35	3.79
9	35	M	None	None	None	+	+	+	0	0.1	0	0	9.47	3.7	24.6
10	16	M	None	None	None	+	−	−	0	0.27	0	0	15.2	7.43	19.1
11	25	M	None	A	None	+	+	+	0	0.14	0	0	5.13	0.42	6.27
12	24	M	None	None	None	+	+	+	0	0	0	0	5.97	6.61	8.84
13	28	M	None	None	None	+	−	−	0	0	0	0	0	0	0.1
14	23	M	None	None	None	+	−	+	0	0	0	0	0.52	0	0.62
15	15	F	None	None	None	+	−	−	0	0.19	0	0	0	0	0
16	20	F	None	None	None	+	+	+	0.46	1.38	0.52	0.5	0.53	0.37	0.5
17	18	M	None	None	Do not like	+	−	−	0	0.19	0	0	0	0	0.1
18	18	F	None	None	A	+	NP	NP	0	0.13	0	0	0.65	0.12	0.42
19	39	F	None	None	OAS	+	−	−	0	0	0	0	2.45	0	0.12
20	31	F	None	None	OAS	+	+	−	0	0.15	0	0	0	0	0
21	18	F	None	None	None	+	+	−	0	0.1	0	0	1.2	0	1.07
22	18	M	None	None	OAS	+	+	+	0	0	0	0	0.38	0.38	0.89
23	26	M	None	None	U	+	−	+	0	0.1	0	0	6.69	1.86	7.43
24	42	F	None	None	OAS	+	+	+	0	0	0	0	7.54	5.6	9.75
25	22	F	None	None	OAS	+	+	+	0	0.1	0	0	0.78	0.26	1.29
26	32	M	None	None	A	+	−	+	0	0.16	0	0	14.4	2.27	13.1
27	21	M	None	None	OAS	+	−	+	0	0.14	0	1.8	0.87	0	0.82
28	52	M	None	OAS	OAS	+	−	+	0	0	0.57	0	26.8	9.09	26.2
29	25	M	OAS	Never eaten	None	+	+	+	0	0	0	0	21.03	12	31
30	23	M	OAS	None	OAS	+	+	+	0	0	0	2.49	25.7	8.18	40
31	33	M	OAS	None	OAS	+	+	+	0	0	0	0	3.19	1.62	4.33
32	26	F	OAS	None	OAS	+	+	+	0	0	0	0	7.21	2.07	9.8
33	28	M	OAS	OAS	U	+	+	+	0.1	0.11	0	0	21.6	6.99	29.3
34	44	F	OAS	OAS	OAS	+	+	+	0	0	0	0	1.75	0.38	2.52
35	25	F	OAS	OAS	OAS	+	+	+	0	0	0	0	2.33	0.57	4.29
36	33	F	OAS	None	U	+	+	+	0	0	0	0	1.5	0.74	4.33
37	35	F	OAS	None	OAS	+	−	+	0	0	0	0	0.19	0.17	0.39
38	26	F	OAS	OAS	U	+	+	+	0	0	0	0	1.89	1.35	4.31
39	38	F	OAS	OAS	U	+	+	+	0	0	0	0	0.11	0	0.14
40	23	F	OAS	None	Never eaten	+	+	+	0	0	0	0	0.49	0.62	0.55
41	28	F	RC	Never eaten	U	+	+	+	0	0	0	0	21.1	11	29.9
42	37	F	U	U	None	+	−	+	0	0	0	0	0.36	0	0.44
43	18	M	U	None	None	+	+	+	0	0	0	0.14	2.73	0.89	24.6
44	24	F	U	U	U	+	+	+	0	0	0	0.11	21	9.74	34.5
45	32	M	U	None	U	+	+	+	0	0	0	0	4.76	0.94	8.71
46	27	M	U	U	U	+	−	−	0	0	0	0	0	0	0
47	27	F	U	None	OAS	+	+	+	0	0	0	0	6.48	2.46	7.83
48	18	F	U	U	U	+	+	−	0	0	0	5.18	0	0	0
49	43	M	U	RC	U	+	+	−	0	0	0	0	7.76	4.39	10.2
50	32	M	U	OAS	OAS	+	+	+	0	0	0	0	6.23	4.99	8.95
51	38	F	U	OAS	OAS	+	+	+	0	0	0	0	2.24	0.65	3.46
52	48	F	U	Never eaten	A	+	+	+	0	0	0.78	0	2.51	1	3.86
53	15	M	U	A	GI	+	+	+	0	0	0	0	11.8	5.91	13.9
54	37	F	U	OAS	GI	+	+	+	0	0	0	0	4.43	1.66	4.69

(Continued)

TABLE E1. *(Continued)*

Subject no.	Age (y)	Sex	Symptoms			SPT response			sIgE level (ImmunoCAP, kU_A/L)						
			Peanut	Hazelnut	Peach	Peanut	Hazelnut	Peach	Ara h 1	Ara h 2	Ara h 3	Ara h 8	Ara h 9	Cor a 8	Pru p 3
55	22	F	U	U	U	+	−	+	0	0	0	0	54.2	14	66.5
56	34	F	U	OAS	U	+	+	+	0	0	0	0	12	3.11	34.7
57	29	M	U	OAS	OAS	+	+	+	0.29	0.39	0.27	0.35	100	67.9	100
58	27	F	U	U	OAS	+	+	+	0	0	0	0.14	8.25	3.29	13.1
59	30	F	U	None	OAS	+	+	+	0	0	0	0	1.17	1.36	4.47
60	37	F	U	OAS	U	+	+	−	0	0	0	0	26.6	1.93	23.9
61	23	F	U	OAS	U	+	+	+	0	0	0	0	35.7	15.4	33.9
62	27	M	GI	None	None	+	+	+	0	0	0	0	47.1	15.9	43.2
63	32	M	GI	None	U	+	+	+	0	0	0	0	1.67	0.1	8.5
64	37	F	OAS	None	U	+	−	+	0	0	0	0	0	0	1.42
65	35	F	A	None	None	+	−	−	6.48	7.07	1.46	0	0	0	0
66	26	F	A	OAS	None	+	+	+	0	0	0	0	2.56	0.71	3.94
67	26	F	A	None	A	+	+	+	0	0	0	0	1.43	0	3.13
68	26	M	A	OAS	OAS	+	+	+	0	0	0	0	3.43	2.29	4.08
69	35	F	A	U	A	+	+	+	0	0	0	0	5.12	1.24	6
70	29	F	A	None	A	+	+	+	0	0	0	0	0.1	0	8.25
71	29	F	A	A	U	+	+	+	0	0	0	0	1.08	0.93	1.43
72	40	F	A	A	A	+	+	+	0	0	0	0	0	0	0
73	39	M	A	None	A	+	+	+	0	0	0	0	9.45	3.08	17.8
74	35	F	A	OAS	U	+	+	+	0	0	0	0	20.7	12.3	25.9
75	38	F	A	None	U	+	+	+	0	0	0	0	27.6	5.98	51.2
76	22	F	A	None	Never eaten	+	+	+	0	0	0	0	12.9	5.15	20.5
77	24	F	A	Never eaten	U	+	+	+	0	0	0	0	12.7	0.18	19.9
78	29	F	A	OAS	U	+	+	+	0	0	0	0	0.52	1.87	1.66
79	28	F	A	None	OAS	+	+	+	0	0	0	0	0.29	0	3.05

A, Anaphylaxis; *F*, female; *GI*, gastrointestinal symptoms; *M*, male; *NP*, not performed; *OAS*, oral allergy syndrome; *RC*, rhinoconjunctivitis; *U*, urticaria.